P. ENT COOPERATION TREAT.

	From the INTERNATIONAL BUREAU			
PCT	* :			
NOTIFICATION OF THE RECORDING OF A CHANGE (PCT Rule 92bis.1 and Administrative Instructions, Section 422) Date of mailing (day month year)	OTTEVANGERS, S., U. Vereenigde Nieuwe Parklaan 97 NL 2587 BN The Hague PAYS-BAS			
17 April 2000 (17.04.00)				
Applicant's or agent's file reference P21796PC00	IMPORTANT NOTIFICATION			
International application No.	International filing date (day month year)			
PCT/NL99.00223	19 April 1999 (19 04 99)			
The following indications appeared on record concerning: the applicant				
Name and Address OTTEVANGERS, S., U. Vereenigde Octrooibureaux Nieuwe Parklaan 97 NL-2587 BN The Hague Netherlands	State of Nationality State of Residence Telephone No. 070-41 66 711 Facsimile No. 070-41 66 799 Teleprinter No.			
2. The International Bureau hereby notifies the applicant that the the person the name X the address.				
Name and Address OTTEVANGERS, S., U. Vereenigde Nieuwe Parklaan 97	State of National ty State of Residence Telephone No.			
NL-2587 BN The Hague Netherlands	070-41 66 711			
Netherlands	070 41 66 799			
	Transfer to			
3. Further observations, financeurs, Please note that the agent's company's name has	s changed.			
4. A copy of the most triation has been present				
X	the control of the co			

od ocenaraje Colombato Mocenaraje Colombato MM Geneva 20 Gwitzeriana

P . ENT COOPERATION TREA.

	From the INTERNATIONAL BUREAU
PCT	
NOTIFICATION OF ELECTION (PCT Rule 61.2)	Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C 20231 ETATS UNIS D'AMÉRIQUE
Date of mailing (day month year) 09 December 1999 (09.12.99)	nitricapalisty as elected Office
International application No. PCT NL99 00223	Applicant's or agent's file reference P21796PC00
International filing date (day month year) 19 April 1999 (19.04.99)	Priority date (day month year) 20 April 1998 (20.04.98)
Applicant	
JANSEN, Gijsbert, Johan et al	
in a notice effecting later election filed with the Inte	ry Examining Authority on: r 1999 (16.11.99)
2 The election X was not was not seemed as the process of the control of the process.	and the second of a supplier of the the second of the decision of the second of the se

in the second of Alberta distribution of the property to Market Report of Market and a

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or	agen	t's file reference	FOR FURTUER ACTION	See Notific	ation of Transmittal of International
P21796PC	00		FOR FURTHER ACTION	Preliminary	Examination Report (Form PCT/IPEA/416)
International	applic	ation No.	International filing date (day/month)	'year)	Priority date (day/month/year)
PCT/NL99	/002	23	19/04/1999		20/04/1998
International C12Q1/68	Paten	t Classification (IPC) or na	tional classification and IPC		
Applicant					
ACADEMI	SCH	ZIEKENHUIS GRON	NINGEN et al.		
1. This int	erna rans	tional preliminary exam mitted to the applicant a	ination report has been prepared according to Article 36.	by this Inte	ernational Preliminary Examining Authority
2. This RI	EPOI	RT consists of a total of	7 sheets, including this cover sl	neet.	
be (se	en ai ee Ri	mended and are the ba	sis for this report and/or sheets on the Administrative Instruction of the	ontaining re	on, claims and/or drawings which have ectifications made before this Authority he PCT).
3. This re	port	contains indications rela	ating to the following items:		
1	\boxtimes	Basis of the report			
11		Priority			
111		Non-establishment of	opinion with regard to novelty, in	ventive step	and industrial applicability
IV		Lack of unity of inventi	ion		
V	\boxtimes	Reasoned statement u	under Article 35(2) with regard to ions suporting such statement	novelty, inv	ventive step or industrial applicability;
VI		Certain documents ci	ted		
VII			international application		
VIII	\boxtimes	Certain observations of	on the international application		

Name and mailing address of the international preliminary examining authority.



European Patent Office

TI 90298 Munich Tull -4 (89.2395)

Fax -49 89 2399 - 4465

Authorized officer

Rang W

Telephone N. +43 ಕೆಕಟಕರ ನಿಮ



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00223

1.	Bas	sis	of	the	report	
----	-----	-----	----	-----	--------	--

1. This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.): Description, pages: as originally filed 1-31 Claims, No.: 24/07/2000 with letter of 24/07/2000 as received on 1-26 Drawings, sheets: as originally filed 1/1 2. The amendments have resulted in the cancellation of: ☐ the description, pages: ☐ the claims, Nos.: ☐ the drawings, sheets:

3.

This report has been established as if (some of) the amendments had not been made, since they have been

considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00223

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes:

Claims 1-26

No:

Claims

Inventive step (IS)

Yes:

Claims 24-25

No:

Claims 1-23, 26

Industrial applicability (IA)

Yes: No: Claims 1-26

Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

ITEM VIII:

Reference is made to the following documents:

D1: EP-A-0 479 117 (HOFFMANN LA ROCHE); 8 April 1992; D2: WO 97 05282 A (UNIV GRONINGEN); 13 February 1997;

NOVELTY 1.

Claims 1-26 meet the requirements of Article 33(2) PCT, because none of the available prior art documents discloses the same combination of features as any of these claims.

INVENTIVE STEP 2.

However, claims 1-23 and 26 do not appear to meet the requirements of Article 33(3) PCT for the following reasons:

2.1 Document D2, which is considered to represent the most relevant state of the art, discloses a method for determining bacteria in a sample, said method comprising the testing of the sample with an oligonucleotide probe by using an in situ hybridization protocol (abstract; page 2, line 25 - page 3, line 16; claims 1-7). Compared to D2, the subject-matter of claim 1 of the present application differs only by the pre-testing of the sample using Gram-staining. The effect of said pretesting is that the in-situ hybridization will be more efficient due to appropriate lysis conditions. Therefore, the technical problem to be solved by present claim 1 may be regarded as how to provide an improved hybridization method for determining bacteria.

It was the satisfies proposed in alaim 1 against he considered as involving an

appear to be linked by a clear technical relationship, since the subject-matter of step (b) is merely defined in terms of the result to be achieved (see item VIII-1.

INTERNATIONAL PRELIMINARY Inter EXAMINATION REPORT - SEPARATE SHEET

below), the interrelationship between steps (a) and (b) of claim 1 cannot be considered as a technical interrelationship. Thus, the method of claim 1 appears to be merely a juxtaposition of known processes functioning in their normal way and not producing any non-obvious working interrelationship (PCT Guidelines IV-8.8 (B1)). Therefore, the method of claim 1 cannot be considered inventive in the sense of Article 33(3) PCT.

- 2.2 The dependent claims 2-23 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step, the reasons being as follows:
 - Clinical samples (claim 2), the determination of the rod or coccus character (i.e. the shape) of the bacteria (claims 4, 9 and 11), rRNA as the template nucleic acid (claims 6, 14 and 18), treatment of the sample with lysozyme (claims 8, 10 and 12), probes capable of hybridizing with nucleic acids from E. faecalis and S. sanguis (claim 13), positive and negative control probes (claim 20), one-step procedures of binding and fixing bacteria simultaneously (claim 22), as well as genera- and species-specific probes (claim 23) are also known from document D2 (abstract; page 3, lines 7-16; page 8, lines 20-26; page 12, lines 13-14; page 14, lines 5-8, 17 and 20-21; figure 1; claims 3, 5-6, 8 and).
 - Blood (claim 3) as a sample is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill.
 - Similarly, the treatment of the sample with lysostaphin or proteinase K (claim 16) is a well-known and obvious selection, which a skilled person regard as a normal option to be included in the method of claim 1 in order to lyse the bacteria.
 - Probes capable of hybridizing with nucleic acids from the bacteria given in claims 5 and 17 are also obvious, because these bacteria straightforward possibilities which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.
 - Finally, the probes having the specific sequences given in claims 7, 15, 19 and 21 cannot be considered inventive (Article 33(3) PCT) because these sequences do

ne phoriai.

- 2.3 Since the method according to claims 1-23 is not inventive (see above), diagnostic test kits according to **claim 26** (comprising means for detecting or identifying bacteria in a sample using said method) also cannot be considered inventive (Article 33(3) PCT).
- 2.4 In contrast, the probe according to **claims 24-25** appears to be inventive (Article 33(3) PCT) for the following reasons:

Document D1, which is considered to represent the closest prior art, discloses probes for detecting or identifying bacteria in a sample, said probe designed to hybridise specifically with nucleic acid in groups of bacterial species (abstract; page 2, lines 34-39; page 3, lines 49-51; page 6, line 55 to page 7, line 5). Compared to said probes of D1, the probe according to **claim 24** differs by its specificity for bacteria with congruent susceptibility or resistance to antibiotics.

Therefore, the problem to be solved by claim 24 may be regarded as how to provide a hybridization probe having specificity for bacteria with congruent susceptibility or resistance to antibiotics. Since the available prior art neither discloses nor suggests such a specificity of hybridization probes, the skilled person would not consider including this feature in the probe according to D1. Consequently, the subject-matter of **claim 24** and its dependent **claim 25** appears to be inventive in the sense of Article 33(3) PCT.

3. INDUSTRIAL APPLICABILITY

The subject-matter of claims 1-26 appears to be industrially applicable in the sense of Article 33(4) PCT.

ITEM VII:

resciosed in the documents in the are not mentioned in the description, and are these documents identified therein.

ITEM VIII:

- 1. Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter in terms of the result to be achieved ("according to an in situ hybridization protocol selected on the basis of [...]") which merely amounts to a statement of the underlying problem. In the absence of the technical features necessary for achieving this result, claim 1 is not clear in the sense of Article 6 PCT.
- 2. The term "probe" used in **claims 1 and 24** is vague and unclear and leaves the reader in doubt as to the chemical nature of such "probe" (oligonucleotide? DNA or RNA?), thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).
- 3. Claims 5, 13, 17, and 24 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("[...] probes capable of hybridising with nucleic acid found in [...]") which merely amounts to a statement of the underlying problem. An analogous objection applies to claim 23 ("[...] probe is selected for its reactivity with [...]"). Thus, the technical features necessary for achieving these results should be added.

	. `	From th		ELIMINARY EXAM	MINING AUTHORITY		Gevenduls, lug	
		To:			CNTY	MORN	PCT	
e ar	TERMUI	VERE Nieuv NL-2	EVANGERS, S. EENIGDE we Parklaan 97 587 BN The Ha G-BAS G. 2000	u. gue NRF2	AMERS	IS 2000 NOTIFICA FOOTHEINTE EXA	TION OF TRANSMITTAL OF RNATIONAL PRELIMINARY AMINATION REPORT (PCT Rule 71.1)	
	Beantwo	ord	bericht gezonden aan			Date of mailing (day/month/year)	01.08.2000	
	def MAP		ant's or agent's file.	eference		IM	IPORTANT NOTIFICATION	
			ational application N NL99/00223	ło.	International filing date (d. 19/04/1999	ay/month/year)	Priority date (day/month/year) 20/04/1998	
		Applic ACAI		(ENHUIS GRO	NINGEN et al.			

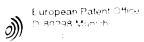
- 1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
- 2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
- 3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.



And the Control of the State of the Control of the State of the State





PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	(Form PCT/ISA/2	of Transmittal of International Search Report 220) as well as, where applicable, item 5 below.
P21796PC00	ACTION	220) do well do. Where applicable, item 5 below.
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/NL 99/00223	19/04/1999	20/04/1998
Applicant		
ACADEMISCH ZIEKENHUIS GRO	NINGEN. et al	
This International Search Report has bee according to Article 18. A copy is being tra	n prepared by this International Searching Aut	hority and is transmitted to the applicant
and the second s	are miles to the international bareau.	
This international Search Report consists		
X It is also accompanied by	a copy of each prior art document cited in this	report.
Basis of the report		
•	international search was carried out on the ba	sis of the international application in the
language in which it was filed. un	less otherwise indicated under this item.	old of the international application in the
the international search w	vas carried out on the basis of a translation of t	he international application furnished to this
Authority (Rule 23.1(b)).		
 b. With regard to any nucleotide an was carried out on the basis of the 	id/or amino acid sequence disclosed in the ir e sequence listing :	nternational application, the international search
contained in the internation	onal application in written form.	
filed together with the inte	ernational application in computer readable form	m.
furnished subsequently to	this Authority in written form.	
	this Authority in computer readble form.	
the statement that the sub- international application a	osequently furnished written sequence listing d is filed has been furnished.	loes not go beyond the disclosure in the
the statement that the info		s identical to the written sequence listing has been
furnished		
2. Certain claims were fou	nd unsearchable (See Box I).	
3 Unity of invention is lac	king (see Box II)	
4 With regard to the title ,		
the text is approved as su		
	hed by this Authority to read as follows:	
METHOD FOR THE KAPID L	DETERMINATION OF BACTERIA	
5. With regard to the abstract.		
Transfer to the aboutour		
as suggested by the applicant fails		None of the figures
because the applicant fails		

INTERNATIONAL SEARCH REPORT

International Application No PCT/NL 99/00223

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12Q1/68 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

H 1 1 1 1 1 1 1 1 1

Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

ENTS CONSIDERED TO BE RELEVANT	
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
EP 0 277 237 A (TORAY INDUSTRIES) 10 August 1988 (1988-08-10) the whole document	1-23
WO 93 24659 A (MICROPROBE CORP) 9 December 1993 (1993-12-09) page 2 - page 3; claim 1	1-23
FR 2 659 981 A (VEF SA) 27 September 1991 (1991-09-27) see abstract; claim 1	1-23
EP 0 479 117 A (HOFFMANN LA ROCHE) 8 April 1992 (1992-04-08) the whole document	1-23
-/	
	EP 0 277 237 A (TORAY INDUSTRIES) 10 August 1988 (1988-08-10) the whole document W0 93 24659 A (MICROPROBE CORP) 9 December 1993 (1993-12-09) page 2 - page 3; claim 1 FR 2 659 981 A (VEF SA) 27 September 1991 (1991-09-27) see abstract; claim 1 EP 0 479 117 A (HOFFMANN LA ROCHE) 8 April 1992 (1992-04-08) the whole document

X Further documents are listed in the continuation of box C	Patent family members are listed in annex
Special categories of cited documents	"T" later document published after the international filing date
All document defining the general state of the lart which is not considered to be of particular relevance.	or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to
"L" document which may throw doubts on priority claim(s) or	involve an inventive step when the document is taken alone
which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance, the claimed invention
O document referring to an oral disclosure, use, exhibition or other means.	cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments such combination being obvious to a person skilled
Politic menticularities of the international form gardinal	in the art

Name and making address of the ISA

European Patent Office IPIB 5818 Patent aan 2

Note: 17 Pis & France | Authorized officer |

European Patent Office IPIB 5818 Patent aan 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 99/00223

	FCI/NL 99/00223
ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
WO 97 05282 A (UNIV GRONINGEN ;WELLING GJALT WIETZE (NL); SCHUT FREDERIK (NL); LA) 13 February 1997 (1997-02-13)	24,25
page 2 - page 3, In 16; pages 12 and 13; claim 7.	1-23
 -	
	1
	WO 97 05282 A (UNIV GRONINGEN ;WELLING GJALT WIETZE (NL); SCHUT FREDERIK (NL); LA) 13 February 1997 (1997-02-13) page 2 - page 3, In 16; pages 12 and 13; claim 7.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 99/00223

	atent document d in search repor	1	Publication date		latent family member(s)	Publication date
EP	0277237	Α	10-08-1988	WO	8800618 A	28-01-1988
WO	9324659	Α	09-12-1993	AU EP US US	4396793 A 0672183 A 5700636 A 5654418 A 5776694 A	30-12-1993 20-09-1995 23-12-1997 05-08-1997 07-07-1998
FR	2659981	Α	27-09-1991	NONE		
EP	0479117	Α	08-04-1992	AU AU CA JP US	657491 B 8489591 A 2052822 A 6090799 A 5620847 A 5635348 A	16-03-1995 09-04-1992 06-04-1992 05-04-1994 15-04-1997 03-06-1997
WO	9705282	Α	13-02-1997	AU EP	6631696 A 0842298 A	26-02-1997 20-05-1998



CIBENIAL CENTRAL

WORLD INTELLECTUAL PROPERTY ORGANIZA International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: C12Q 1/68, 1/04

Al

(11) International Publication Number:

WO 99/54502

(43) International Publication Date:

28 October 1999 (28,10,99)

(21) International Application Number:

PCT/NL99/00223

(22) International Filing Date:

19 April 1999 (19.04 99)

(30) Priority Data:

98201253.6

20 April 1998 (20.04.98)

ΕP

(71) Applicants (for all designated States except US): ACADEMISCH ZIEKENHUIS GRONINGEN [NL/NL]: Oostersingel 59, NL-9713 EX Groningen (NL). RIJK-SUNIVERSITEIT TE GRONINGEN (NL/NL); Broerstraat

5, D-9712 CP Groningen (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): JANSEN, Gijshert, Johan [NL/NL]; Suderspaerlan 16, NL-9061 BJ Giekerk (NL). DEGENER, John, Edward [NL/NL]; de Rozentuin 1, NL-9203 LP Drachten (NL). WELLING, Gjalt, Wietze [NL/NL]; Hoofdstraat 48, NL-9315 PC Roderwolde (NL).

(74) Agent: OTTEVANGERS, S., U.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG. KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK. SL. TJ, TM, TR. TT, UA, UG, US, UZ, VN, YU, ZA ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU. TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES. FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

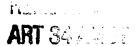
With international search report.

(54) Title: METHOD FOR THE RAPID DETERMINATION OF BACTERIA

(57) Abstract

The invention relates to the detection, identification and diagnosis of bacteria in samples in general and in particular in clinical samples such as blood, urine, saliva, cerebrospinal fluid that are taken from patients that are possibly infected with a, as yet, unknown, possibly pathogenic bacterium, or during follow-up diagnostic testing to, for example, evaluate therapeutic measures that have been taken so far to treat the disease. The invention provides a method for detecting or identifying a bacterium suspected of being present in a sample comprising testing said sample by Gram-staining and testing said sample with a probe according to an in situ hybridisation protocol selected on the basis of the outcome of said Gram-staining. The invention also provides probes for use in said method.

WO 99/54502



CLAIMS

- A method for determining a pacterium suspected of being present in a sample comprising
- a) testing said sample by Gram-staining and b) testing said sample with a probe according to an in
- situ hybridisation protoccl selected on the basis of the. outcome of said Gram-staining.
 - 2. A method according to claim 1 wherein said sample is a clinical sample.
 - 3. A method according to claim ? wherein said sample is mammalian blood, preferably being derived from a human.
 - 4. A method according to claim 1, 2 or 3 wherein said Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
- 5. A method according to claim 4 wherein said character is of the rod type, further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in Escherichia coli, in Klebsiella pheumoniae, in Klebsiella
- oxytoca, in Serratia marcescens, in Enterobacter 20 aerogenes, in Enterobacter cloacae, in Proteus vulgaris, in Proteus mirabilis, in Salmonella typhi, in Pseudomonas aeruginosa.
 - 6. A method according to claim 5 wherein said nucleic acid is ribosomal RNA.
 - T. A method according to claim 6 wherein said probe is having no more than five, presurably no more than two mismatches with a probe selected of a group composed of probes having a sequence SCCTSCCAGTTTCGAATG or
- CTAGCCCTACTCGTAAGG or GAGCHAAGGTATTAACTTTACTCCC or

WO 99/54502

25

PCT/NL99/00223

sample to treatment with a lysis buffer comprising lysozyme.

- 9. A method according to claim 1, 2 or 3 wherein said Gram-staining indicates the presence of a Gram-positive
- 5 bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
 - 10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.
 - ll. A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.
 - 12. A method according to claim 11 wherein said character
- is chain-like, further comprising subjecting said sample to treatment with a lysis bufter comprising lysozyme.
 - 13. A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic
- 20 acid found in Enterococcus faecalis, in Streptococcus pneumoniae, in Streptococcus mitis, in Streptococcus viridans, in Streptococcus sanguis, in Enterococcus faecium.
 - 14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.
 - 15. A method according to claim 14 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG or
- AGAGAAGCAAGCTTCTCGTUCG or GCCACTCCTCTTTTCCGG.

 16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase F.

WO 99/54502

TEREST SEE SET SERVICE

PCT/NL99/00223

from a group of probes capable of hybridising with nucleic acid found in Staphylococcus aureus, in Staphylococcus haemolyticus, in Staphylococcus saprophyticus.

- 18. A method according to claim 17 wherein said nucleic acid is ribosomal RNA.
- 19. A method according to claim 18 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence GCTAATGCAGCGCGGATCC or
- 10 CCGAAGGGGAAGGCTCTA or AGAGAAGCAAGCTTCTCGTCCGTT.

 20. A method according to any of claims 4 to 19 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.
- 21. A method according to claim 20 wherein said positive control probe comprises no more than five mismatches with a probe with the sequence GCTGCCTCCCGTAGGAGT and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence
- 20 ACTCCTACGGGAGGCAGC.
 - 22. A method according to anyone of claims 1 to 21 further comprising a one-step procedure to bind bacteria present in said sample to a microscopic slide and simultaneously fix intracellular structures.
- 23. A method according to anyone of claims 1 to 22 wherein said probe is selected for its reactivity with one or a group of bacterial genera and/or species having congruent suscept:bility to antibiotic treatment.
- 24. A probe detecting or identifying a bacterium in a sample, preferably a chirical sample, said probe capable of hybridising with nucleic acid found in a group of bacterial general and/or "cup) species having congruent susceptibility to antipictic treatment.
 - 25 A probe addongs no sold and to see the second of the se

probes lying a sequence GCCTGCCAGTTTCGAATG or GTAGCCCTACTCGTAAGG or GAGCAAAGGTATTAACTTTACTCCC or GTTAGCCGTCCCTTTCTGG or TTATCCCCCTCTGATGGG or AGAGAAGCAAGCTTCTCGTCCG or GCCACTCCTCTTTTTCCGG or

- 5 GCTAATGCAGCGCGGATCC or CCGAAGGGGAAGGCTCTA or AGAGAAGCAAGCTTCTCGTCCGTT.
 - 26. A diagnostic test kit comprising means for detecting or identifying a bacterium suspected of being present in a sample using a method according to anyone of claims 1 to
- 10 23 or using a probe according to claim 24 or 25.